Post-transcriptional regulation of cytokine mRNA controls the initiation and resolution of inflammation.

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Abbreviations:

ARE, adenine- and uridine-rich element; AUF1, ARE/poly-(U) binding degradation factor 1; Bcl-2, B-cell lymphoma-2; β-TrCP, β-transducin repeat–containing protein; CCL2, CC-chemokine ligand 2; C/EBPβ, CCAAT/enhancer-binding protein β; COX-2, cyclooxygenase 2; CXCL1, CXC-chemokine ligand 1; DCs, dendritic cells; ERK, the extracellular signal-regulated kinase; GAIT complex, the interferon-γ-activated inhibitor of translation complex; γ-GCSh, γ-glutamylcysteine synthetase heavy subunit; GM-CSF, granulocyte-macrophage colony-stimulating factor; GVHD, graft-versus-host disease; hnRNP D, heterogeneous nuclear ribonucleoprotein D; hnRNP L, heteronuclear ribonucleoprotein L; hnRNP U, heterogeneous nuclear ribonucleoprotein U; HuR, embryonic lethal abnormal vision system human homologue 1 (ELAV1); IL, interleukin; IκB, inhibitor of transcription factor NF-κB; IKK, IκB kinase; iNOS, inducible nitric oxide synthase; IRFs; IFN-regulatory factors; JNK, the c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; Mcl-1, myeloid cell leukemia-1; miRNAs, microRNAs; MKP-1, MAPK phosphatase-1; MEFs, embryonic fibroblasts; NLRs, Nod-like receptors; TGF-β, transforming growth factor-β; TIA-1, T-cell-restricted intracellular antigen-1; TIAR, TIA-1-related protein; TLRs, Toll-like receptors; TNF, tumor necrosis factor; TTP, tristetraproline; PAMPs, pathogen-associated molecular patterns; PRRs, pattern-recognition receptors; RBPs, RNA-binding proteins; Regnase-1, regulatory RNase-1; RISC, RNA-induced silencing complex; RLRs, helicase RIG-I-like receptors; RRMs, RNA recognition motifs; SIRT1, sirtuin 1; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; VEGFA, vascular endothelial growth factor A.

Keywords: post-transcriptional regulation; inflammation; cytokine mRNA stability; regnase-1 (Zc3h12a, Mcpip1)

Abstract

Cytokines are critical mediators of inflammation and host defense. Cytokine production is regulated during transcription and post-transcription. Post-transcriptional regulation modifies mRNA stability and translation, allowing for the rapid and flexible control of gene expression, which is important for coordinating the initiation and resolution of inflammation. We review here a variety of post-transcriptional control mechanisms that regulate inflammation and discuss how these mechanisms are integrated to coordinate this essential process.

Introduction

Inflammation is an essential component of the immune responses that allows multicellular organisms to remove detrimental stimuli and repair tissue damage. Causes of inflammation include various factors such as microbial infection, tissue injury and cellular stress. Inflammation is classically characterized by five symptoms: redness, swelling, heat, pain, and loss of tissue function. Inflammatory responses are rapidly elicited in response to infection with various pathogens or cellular stimuli (Lawrence *et al.*, 2002). Proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6 mediate inflammation. In resting cells of the innate immune system the expression of cytokines is suppressed, only being induced rapidly in response to infection by pathogens via a set of pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and Nod-like receptors (NLRs) (Takeuchi and Akira, 2010; Beutler, 2009; Medzhitov and Horng, 2009). PRRs recognize molecular structures that are broadly shared by pathogens, known as pathogen-associated molecular patterns (PAMPs), such as bacterial lipopolysaccharide (LPS) (Janeway, 1989; Akira *et al.*, 2006). Upon PAMP recognition, PRRs initiate a series of signaling pathways that lead to the activation of transcription factors, including NF-κB, activator protein 1 (AP-1), interferon (IFN)-regulatory factors (IRFs), and CCAAT/enhancer-binding protein β (C/EBPβ). In addition to eliciting inflammation, PRR signaling simultaneously induces maturation of dendritic cells (DCs), which are responsible for stimulating the second line of host defense, adaptive immunity.

Activated transcription factors transcribe a family of mRNAs encoding proinflammatory cytokines that are translated to proteins, resulting in the secretion of cytokines. Once a factor causing inflammation has been neutralized, the cytokine-mediated inflammatory response is rapidly terminated and damaged tissues are repaired. However, overproduction of cytokines (cytokine storm) by immune cells to overwhelm pathogens can be fatal. A cytokine

storm can also be caused by noninfectious disorders such as graft-versus-host disease (GVHD). Inflammatory responses are also critical for the pathogenesis of autoimmune diseases (Marshak-Rothstein, 2006).

The expression of cytokines and pro-inflammatory factors is tightly regulated at multiple levels, including gene transcription, mRNA translation, and mRNA degradation (Stoecklin *et al*., 2006). Transcriptional regulation has been characterized and shown to control the expression of cytokines. Although transcription is the essential first step in the regulation of gene expression, it is a complicated process that cannot be rapidly stopped or redirected. These regulatory pathways are tightly controlled by multiple biological networks, in particular, post-transcriptional regulation, which determines the fate of mRNA in association with RNA-binding proteins (RBPs) such as tristetraprolin (TTP), ARE/poly-(U) binding degradation factor 1 (AUF1), ZCCHC11, and Regnase-1 (also known as Zc3h12a, Mcpip1) (Anderson, 2010; Palanisamy *et al.*, 2012). In addition to RBPs, microRNAs (miRNAs) are important regulators of gene expression in the immune system through cooperation with RBPs. Post-transcriptional regulation controls mRNA stability and translation and can rapidly repress protein expression despite on-going gene transcription. Thus, given its ability to rapidly control gene expression, post-transcriptional dampening of protein expression can actively promote the resolution of inflammation to prevent unintended tissue damage (Stoecklin and Anderson, 2006). In this way, post-transcriptional control mechanisms link the initiation phase and the resolution phase of inflammation. Most post-transcriptional control mechanisms target the 3′-untranslated region (3′-UTR) of mRNAs to repress expression of the target transcript (Anderson, 2008; von Roretz and Gallouzi, 2008; Shyu *et al*., 2008). In this review, we focus on post-transcriptional regulation mechanisms that regulate mRNA stability and modulate both the initiation and resolution of inflammation. We also discuss how different types of post-transcriptional control mechanisms coordinate to regulate immune-mediated inflammation.

AU-rich Elements (AREs) are Critical in the Regulation of Cytokine mRNA Stability

The regulation of mRNA stability and translation is important for the control of gene expression, and is determined by *cis*-acting sequences in the 3′-UTRs that promote mRNA degradation and suppress mRNA translation. A well-characterized motif present within the 3′-UTR of mRNA is the adenine- and uridine-rich element (ARE). Clusters of AREs found in mRNAs encoding cytokines were first identified over 25 years ago (Caput *et al.*, 1986) and subsequent studies showed that cytokines, chemokines, lymphocytes, proto-oncogenes, and pro-inflammatory genes are subject to ARE-mediated decay (Shaw and Kamen, 1986; Hamilton *et al.*, 2007). AREs provide binding sites for *trans*-acting RBPs, such as TTP, AUF1, and HuR (embryonic lethal abnormal vision system human homologue 1 (ELAV1)), that subsequently regulate the stability and translation of mRNA. ARE basic motifs include pentamers of AUUUA, nonamers of UUAUUUAUU, and AU-rich clusters composed of linked pentamers and/or nonamers (Wilusz *et al.*, 2001). Bakheet *et al.* created a database of human ARE mRNAs (ARED; http://brp.kfshrc.edu.sa/ARED/) based on the patterns of AUUUA motifs (Bakheet *et al.*, 2006) demonstrating approximately 8% of mRNAs within the human genome contain AREs. AREs are located at the 3′-UTR of mRNA transcripts for cytokines such as *granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF, IL-2, IL-3, IL-6,* and *IL-8,* as well as pro-inflammatory factors like cyclooxygenase 2 (COX-2) (*Figure 1*). mRNAs harboring AREs are prone to quick degradation in innate immune cells in general, although stimulation with LPS can extend their half-life (Hao and Baltimore, 2009). Thus, AREs are critical elements in controlling gene expression at the post-transcriptional level.

Interaction of Cytokine mRNAs with RNA Binding Proteins

Cytokine mRNA expression is restricted in resting cells through continuously active mRNA

decay mechanisms. Induction of mRNA decay pathways allow attenuation of cytokine production through interactions with RBPs (Anderson, 2009). AREs in 3′-UTR of mRNAs promote binding of RBPs that degrade or stabilize mRNA transcripts, often in association with other proteins. Several studies have identified RBPs that can bind to AREs and regulate mRNA stability and translation, including TTP and AUF1. Cytokine mRNAs and their functional interactions with important RBPs are summarized in *Table 1*. A detailed description of each RBP and their association with cytokine mRNAs are discussed next.

Tristetraprolin (TTP)

Tristetraprolin is a well-characterized zinc-finger protein that regulates immune functions (Sanduja *et al.*, 2011). TTP transcription is induced by inflammatory modulators such as TNF, LPS (Chen *et al.*, 2006), glucocorticoids (Smoak and Cidlowski, 2006), insulin (Cao *et al.*, 2008), and IFN-γ (Sauer *et al.*, 2006). TTP-deficient mice develop arthritis, dermatitis, and cachexia, a syndrome caused by the spontaneous overexpression of pro-inflammatory cytokines, particularly TNF (Taylor *et al.* 1996; Carballo and Blackshear, 2001) suggesting TTP is involved in the damping of inflammation. Treatment of these mice with TNF-specific antibodies reversed almost all pathology, implicating dysregulated cytokine expression as a principal cause of this phenotype. Subsequent biochemical studies showed that TTP decreased *TNF* mRNA stability by binding to its ARE (Carballo *et al.*, 1998). TTP harbors two Cys-Cys-Cys-His (CCCH)-type zinc-finger domains and associates with mRNA via AU-rich elements present in the 3′-UTR, leading to removal of the poly(A) tail by recruitment of a deadenylase (Carrick *et al.*, 2004).

Deadenylation promotes rapid mRNA degradation that is thought to occur during processing of (P)-bodies, small cytoplasmic foci that contain many enzymes required for mRNA

decay. Cells exposed to various stresses such as heat shock, oxidative stress, or glucose deprivation promote the assembly of stress granules, small cytoplasmic foci that harbor translationally arrested mRNAs, stalled translation initiation factors, and ARE-binding proteins such as T-cell-restricted intracellular antigen-1 (TIA-1) and TIA-1-related protein (TIAR) (Anderson and Kedersha, 2008). Under conditions of stress, TTP is recruited to stress granules (Murata *et al.*, 2005; Kedersha *et al.*, 2005), and facilitates delivery of selected mRNAs from stress granules to P-bodies for degradation, suggesting a TTP-dependent dynamic relationship between stress granules and P-bodies. TTP also destabilizes mRNA transcripts encoding multiple inflammatory modulators, including GM-CSF (Carballo *et al.*, 2000), IL-2 (Ogilvie *et al.*, 2005), IL-6 (Sauer *et al.*, 2006), c-Fos (Chen *et al.*, 2001), inducible nitric oxide synthase (iNOS) (Linker *et al.*, 2005), COX-2 (also known as PTGS2) (Phillips *et al.*, 2004), CC-chemokine ligand 2 (CCL2) (Sauer et al., 2006), CCL3 (Sauer *et al.*, 2006), CXC-chemokine ligand 1 (CXCL1) (Datta et al., 2008), IFN-γ (Ogilvie *et al.*, 2009), and IL-10 (Stoecklin *et al.*, 2008). Although the exact mechanism of cytoplasmic TTP-dependent mRNA turnover remains unclear, various models have been proposed, including targeting of mRNA to the P-body. Together, these studies demonstrate TTP is a key player in post-transcriptional gene regulation, particularly with regard to the regulation of ARE-mediated decay of cytokine mRNAs.

ARE/poly-(U) Binding Degradation Factor 1 (AUF1, also known as hnRNP D) and Heterogeneous Nuclear Ribonucleoprotein U (hnRNP U)

AUF1 (also known as heterogeneous nuclear ribonucleoprotein D, hnRNP D) is an established attenuator of inflammatory cytokine responses that acts by destabilizing inflammatory cytokine mRNAs, such as *IL-2*, *TNF,* and *IL-1β,* and other mRNAs containing AREs in 3′-UTRs (Lu *et*

al., 2006; Sadri and Schneider, 2009). AUF1 is an ARE-binding protein that consists of four highly related protein isoforms generated by alternate RNA splicing from a single genetic locus (Guhaniyogi and Brewer, 2001). The four AUF1 isoforms contain two RNA recognition motifs (RRMs) in the central portion of the protein (Wagner *et al.*, 1998; Barker *et al.*, 2012). Mice lacking AUF1 develop chronic dermatitis with increasing age characterized by pruritis and excoriations, which are associated with increased IL-2, TNF-α, and IL-1β (Sadri and Schneider, 2009). Histological analysis of AUF1-deficient mice showed marked epidermal acanthosis and spongiosis, neovascularization, and elevated numbers of inflammatory cells, including T cells, macrophages, neutrophils, mast cells, and eosinophils. Although AUF1 is primarily localized in the nucleus, it shuttles to the cytoplasm and attaches to ARE-containing mRNAs through MAPK phosphatase-1 (MKP-1)-induced translocation (Yu *et al*., 2011). MKP-1 functions as a negative regulator of the host inflammatory response to infection by specific dephosphorylation of activated mitogen-activated protein kinases (MAPKs), which when activated induces pro-inflammatory cytokines. MKP-1 promotes translocation of AUF1 from the nucleus to the cytoplasm in response to LPS stimulation, resulting in destabilized cytokine mRNAs. Although the mechanisms underlying the regulation of cytokine mRNA stability by AUF1 have not been fully delineated, these findings show the complexity of the processes that control AUF1-regulated cytokine mRNA expression and cellular localization.

Recently, another member of the hnRNP family,, heterogeneous nuclear ribonucleoprotein U (hnRNP U), was shown to regulate TLR-induced proinflammatory cytokine production in macrophages (Zhao *et al.*, 2012). Whereas AUF1 (hnRNP D) is principally associated with destabilization of proinflammatory cytokine mRNAs, hnRNP U stabilizes cytokine mRNAs such as *TNF-α, IL-6,* and *IL-1β*, but not *IL-12*. hnRNP U expression is induced by TLR signaling and binds to cytokine mRNAs through an Arg-Gly-Gly (RGG) domain. Moreover, TLR stimulation induces translocation of hnRNP U from the nucleus to the cytoplasm. These studies indicate that hnRNP proteins have different functions in the regulation

of proinflammatory cytokine production by either increasing or decreasing mRNA stabilization. However, the functional relationship between AUF1 (hnRNP D) and hnRNP U in the regulation of mRNA stability requires further investigation.

ZCCHC11

In contrast to the normal role of zinc-finger proteins in dampening the expression of inflammatory mediators, the zinc-finger protein ZCCHC11 (also known as terminal uridylyltransferase 4) stabilizes mRNA encoding *IL-6* (Jones *et al.*, 2009). ZCCHC11 is a CCHC-type zinc-finger domain containing RNA-binding protein with a nucleotidyltransferase domain. ZCCHC11 has uridyltransferase activity and can add non-genome-encoded uridine residues to the 3′ ends of RNA (Jones *et al.*, 2009). Targeted knockdown of ZCCHC11in the A549 human alveolar epithelial cell line inhibited TNF-induced secretion of IL-6, CCL5, transforming growth factor-β (TGF-β) and vascular endothelial growth factor A (VEGFA). Decreased expression of IL-6 was caused by increased decay of *IL-6* mRNA transcripts, indicating that ZCCHC11 stabilizes *IL-6* mRNA. A recent study found that ZCCHC11 adds terminal uridine to microRNA, miR-26, which target *IL-6* mRNA (Jones *et al.*, 2009). Uridylated miR-26 fails to repress *IL-6* mRNA, and ZCCHC11 thereby potentiates IL-6 production in response to TNF stimulation. Although this mechanism of post-transcriptional control provides new insight to the regulation of cytokine mRNA stability, it is unclear which other miRNAs are subject to uridine modification and how this process determines the levels of active miRNAs. Confirmation of this mechanism will provide further insight to post-transcriptional regulation and miRNA function.

Regulatory RNase-1 (Regnase-1, also known as Zc3h12a, Mcpip1)

RNase Regnase-1 (also known as Zc3h12a, Mcpip1) is composed of a PilT-N terminal (PIN)-like RNase domain and a CCCH-type zinc-finger domain (Matsushita *et al*., 2009) and is critical in preventing autoimmunity by controlling the stability of mRNA encoding IL-6, IL-12p40, and Regnase-1 itself (*Figure 2*). The production of IL-6 and IL-12p40 in response to TLR ligands is greater in *Regnase-1*-deficient macrophages compared with normal macrophages (Matsushita *et al*., 2009). The decay of *IL-6* mRNA is impaired in *Regnase-1*-deficient macrophages, as Regnase-1 destabilizes mRNA via a conserved element, but not AREs, present in the 3′-UTR of *IL-6* mRNA. *Regnase-1*-deficient mice spontaneously develop severe autoimmune inflammatory disease, indicating Regnase-1-mediated control of mRNA expression has an essential role in maintaining homeostasis.

Although the expression of *Regnase-1* mRNA is induced in response to stimulation with TLR ligands, Regnase-1 protein is expressed even in unstimulated macrophages, mouse embryonic fibroblasts (MEFs), thymus, spleen, lymph nodes, and lungs (Iwasaki *et al.*, 2011). Regnase-1 protein is rapidly degraded in response to stimulation with IL-1β or TLR ligands but not TNF. Degradation of Regnase-1 protein is important for higher expression of *IL-6* mRNA. Biological studies have shown that inhibitors of transcription factor NF-κB (IκB) kinase (IKK) complex (an enzyme involved in the activation of transcription factor NF-κB by phosphorylation and ubiquitin-proteasome-mediated degradation of NF-κB-inhibitory protein IκBα, and composed of IKKα, IKKβ, and IKKγ (NEMO) subunits) controls the stability of *IL-6* mRNA by phosphorylating Regnase-1 in response to stimulation via IL-1R or TLR. Regnase-1 is phosphorylated by IKKα/β, and phosphorylated Regnase-1 undergoes ubiquitin-proteasome-mediated degradation via the E3 ligase β-TrCP complex (β-transducin repeat–containing protein; also known as FBW1). Regnase-1 is then re-expressed in IL-1R- or TLR-activated cells after a period of lower expression. *Regnase-1* mRNA is negatively

regulated by Regnase-1 itself via a stem-loop region present in the *Regnase-1* 3′-UTR. These finding show that the IKK complex phosphorylates IκBα, thereby activating transcription, and Regnase-1, thereby releasing a 'brake' on *IL-6* mRNA expression (*Figure 2*). Although it remains to be determined which *cis*-acting and *trans*-acting elements are responsible for specific Regnase-1-mediated mRNA destabilization, the regulatory mechanisms can suppress unwanted inflammation and rapid production of proinflammatory cytokines in response to infection.

miRNA-Mediated Regulation of Cytokine Expression Levels

miRNAs are a specific class of evolutionarily conserved small (19-25 nucleotides) endogenous non-coding RNAs that regulate gene expression at the post-transcriptional level (Rana, 2007). miRNAs use base-pairing to bind to partially or perfectly complementary sites in the 3′-UTR of mRNAs, to induce translational repression or mRNA degradation of the target gene (Rana, 2007) (*Table 2*). Thus, miRNAs regulate diverse cellular and molecular processes, including cellular proliferation, differentiation, apoptosis and innate immunity (Rana, 2007; Friedman *et al*., 2009). Regulation of cytokine expression through the association of miRNAs with RBPs can fine-tune immune responses by a number of mechanisms including: (1) mRNA decay and translational inhibition through miRNA and RBP cooperation; (2) mRNA stabilization due to competition between RBPs and miRNAs; and (3) environmental effects on mRNA stability, mediated through miRNAs and RBPs. An early example of miRNA-assisted RBP regulation of cytokine mRNA was observed for TNF-α, where miR-16 had a partial sequence match with the *TNF-α* ARE and ARE-mediated decay of *TNF-α* was dependent on both TTP and miR-16 (Jing *et al.*, 2005). Based on these data, it was hypothesized that miR-16-bound RNA-induced silencing complex (RISC) assisted TTP binding to ARE, which subsequently induced mRNA degradation by deadenylation. Recently, three miRNAs, miR-125b, miR-221, and miR-579,

were up-regulated during LPS-induced tolerance (a state that causes *TNF-α* mRNA to be degraded), and were capable of either associating with TTP to accelerate *TNF-α* mRNA decay or blocking TNF-α translation, possibly through recruitment of the translational inhibitor TIAR (El Gazzar and McCall, 2010).

Alternatively, HuR binding to *c-Myc* 3′-UTR repressed c-Myc expression by recruiting let-7/RISC to an adjacent site on the *c-Myc* 3′-UTR (Kim *et al*., 2011). HuR is an ARE-binding protein that modulates the expression of ARE-containing transcripts encoding immune modulators such as IL-4, TNF, IFN-γ, and COX-2. HuR is ubiquitously expressed in mammalian tissues, and although predominantly located in cell nuclei (>90% of the total), transient shuttling between nucleus and cytoplasm occurs (Barker *et al.*, 2012). HuR contains three RRMs, with a long hinge region that includes a domain responsible for nuclear/cytoplasmic shuttling, separating the second and the third RRM. Phosphorylation of serine residues (S202 or S242) within this hinge region influences subcellular localization of HuR, leading to an accumulation of HuR in the nucleus. Domains 1 and 2 of RRM mediate recognition of U-rich target RNA sequences, whereas domain 3, originally implicated in binding poly-A tails of mRNAs, mediates 30-terminal adenosylation of non-polyadenylated RNA. Although HuR typically promotes mRNA expression for p21, proto-oncogene C-FOS, VEGF, MKP-1, iNOS, GM-CSF, sirtuin 1 (SIRT1), TNF-α, B-cell lymphoma-2 (Bcl-2), myeloid cell leukemia-1 (Mcl-1), COX-2, γ-glutamylcysteine synthetase heavy subunit (γ-GCSh), urokinase-type plasminogen activator (uPA) and its receptor (uPAR), p53, IL-3, and cyclins A2, B1, E1, and D1 (Abdelmohsen and Gorospe, 2010) through mRNA stabilization, HuR can interact with let-7 miRNA to repress the expression of *c-Myc* transcripts (Kim *et al*., 2011).

Conversely, RBPs and miRNAs can also compete with each another to promote mRNA stability. miR-4661, which contains a seed region that is complementary to ARE sequences, can up-regulate both *IL-10* mRNA and protein levels upon transfection into LPS-stimulated RAW264.7 macrophages (Ma *et al*., 2010). Therefore, miR-4661 competes with

TTP for binding to ARE sequences in *IL-10* mRNA, thus protecting the mRNA from TTP-mediated degradation. These studies show that ARE-binding proteins can interact with miRNA machinery to modulate gene expression at the post-transcriptional level.

Conclusions

Post-transcriptional regulation is critical for inflammatory disease progression by controlling the production of cytokines, which activate multiple signaling cascades in inflammation, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), NF-κB, and p38 MAPK. In most cases, post-transcriptional control mechanisms dampen the expression of inflammatory mediators by promoting mRNA decay or by inhibiting protein translation. These effects are mediated by regulatory factors that bind to *cis*-acting elements in 3′-UTRs of mRNA transcripts. The most common regulatory element is ARE. Loss of post-transcriptional regulation of cytokine mRNAs can dramatically increase cytokine production, leading to tissue destruction and increased mortality. Given the onset of cytokine production, several features of post-transcriptional control play a critical role in their maintenance. However, these mechanisms are complex and remain unclear. A greater understanding of how RBPs regulate mRNA stability could potentially lead to improved therapies for inflammatory diseases.

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Figure legends

Figure 1: Schematic diagram of cytokine mRNA and sites of post-transcriptional regulation.

Inflammatory cytokines are regulated post-transcriptionally through 5′- and 3′-UTRs. The 5′-UTR dictates mRNA translation initiation, whereas the 3′-UTR regulates mRNA turnover. Cytokine mRNA stability and translation are highly regulated via their AU-rich elements (AREs). *IL-6, TNF-α,* and *COX-2* AREs are underlined.

Figure 2: Post-transcriptional regulation of IL-6 expression by Regnase-1.

Regnase-1 destabilizes *Il6* mRNA post-transcriptionally. In response to IL-1R or TLR stimulation, the IKK complex phosphorylates IκBα and Regnase-1, inducing ubiquitin proteasome-mediated degradation. This regulation facilitates the rapid and robust production of IL-6 protein.

Tables

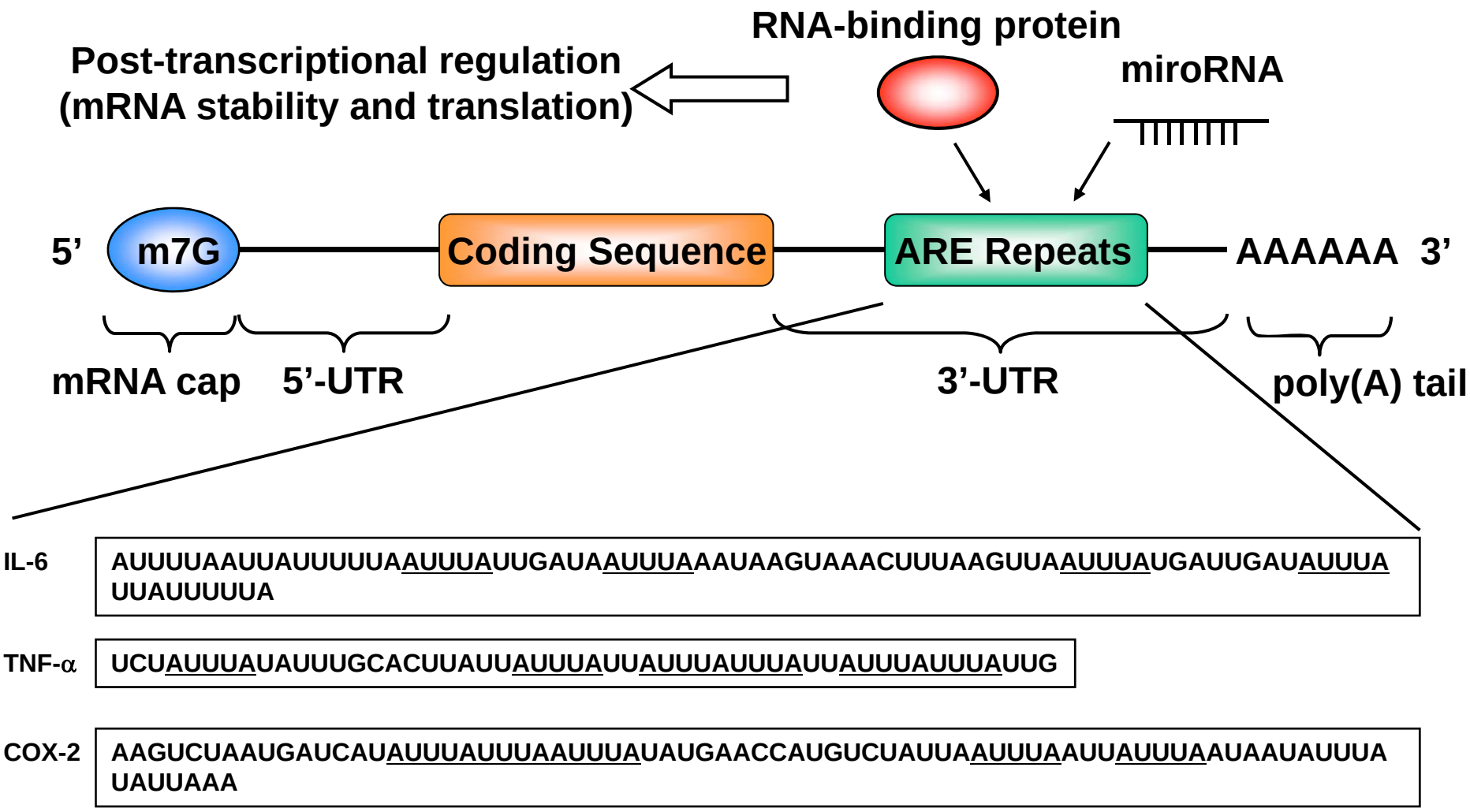
Table 1: RBP Regulation of Specific Cytokine mRNAs

Cytokine mRNAs	RNA-binding protein	Regulation
IL-1β	TTP	mRNA destabilized
	AUF1	mRNA destabilized
IL-2	TTP	mRNA destabilized
	NF90	mRNA stabilized
IL-3	TTP	mRNA destabilized
IL-4	HuR	mRNA stabilized
IL-6	Regnase-1	mRNA destabilized
	TTP	mRNA destabilized
	AUF1	mRNA destabilized
IL-10	TTP	mRNA destabilized
	AUF1	mRNA destabilized
IL-12p40	Regnase-1	mRNA destabilized
TNFα	TTP	mRNA destabilized
	AUF1	mRNA destabilized
	TIA1	Translational repression
	FXR1	Translational repression
	HuR	Translational repression
GM-CSF	TTP	mRNA destabilized
IFNγ	GAIT complex	Translational repression
	TTP	mRNA destabilized
	HuR	mRNA stabilized

Table 2: Cooperative functions between RBPs and miRNAs in the repression and activation of shared target cytokine mRNAs

Cytokine mRNAs	RNA-binding proteins	miRNAs
TNFα	TTP	miR-16
	TTP and TIAR	miR-125, -221, or -579
	FXR1 and AGO2	miR-369-3
IL-10	TTP	miR-4661
VEGF	hnRNP L	miR-297, or -299

Figure 1


RNA-binding protein
Post-transcriptional regulation
(mRNA stability and translation)
miroRNA
5’
m7G
Coding Sequence
ARE Repeats
AAAAAA 3’
mRNA cap
5’-UTR
3’-UTR
poly(A) tail
IL-6
AUUUUAAUUAUUUUUAAUUUAUUGAUAAUUUAAAUAAGUAAACUUUAAGUUAAUUUAUGAUUGAUAUUUA
UUAUUUUUA
TNF-α
UCUAUUUAUAUUUGCACUUAUUAUUUAUUAUUUAUUUAUUAUUUAUUUAUUG
COX-2
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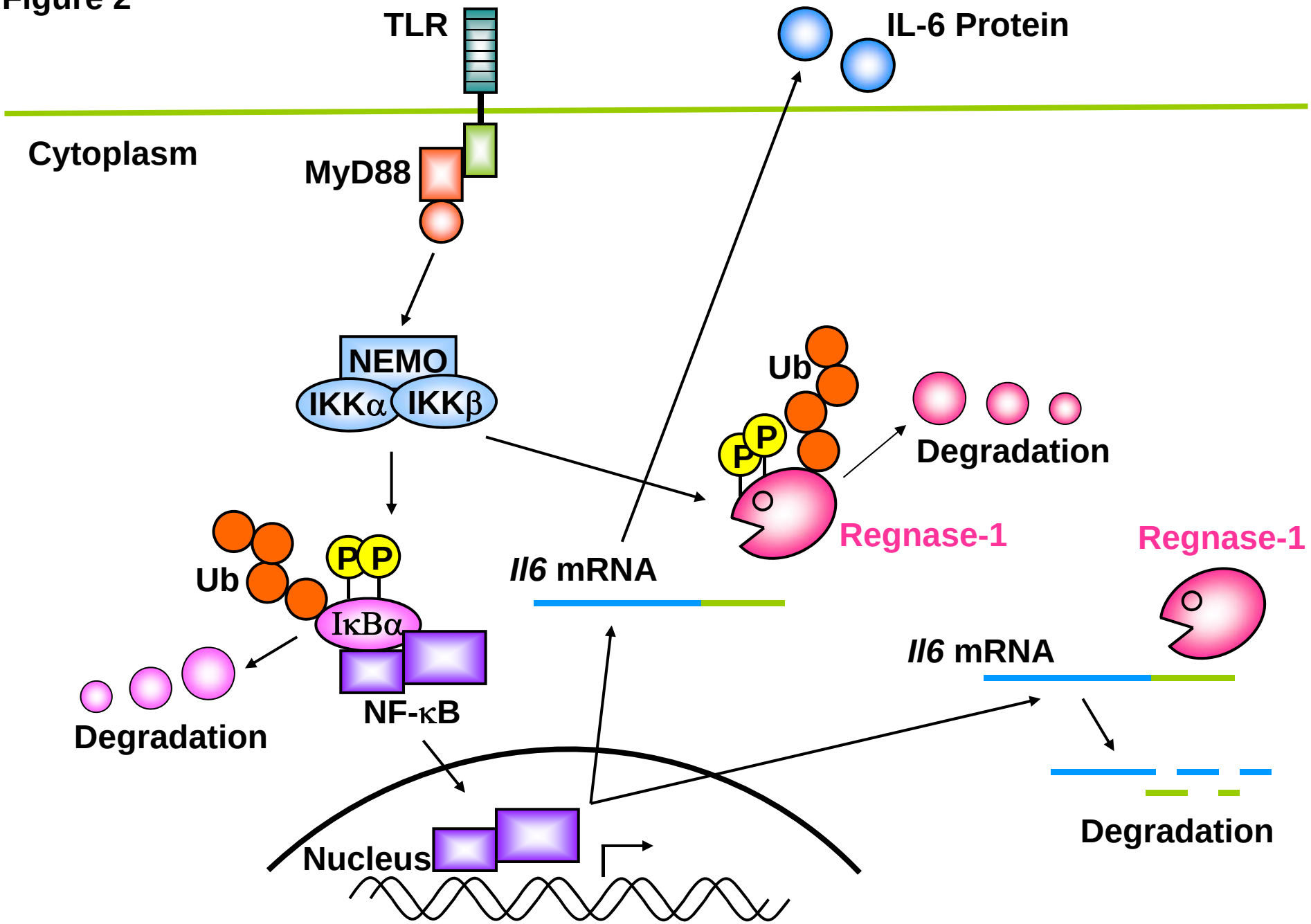
UAUUAAA

Figure 2


TLR
IL-6 Protein
Cytoplasm
MyD88
NEMO
IKKα
IKKβ
Ub
P
P
Degradation
Regnase-1
Regnase-1
Il6 mRNA
Ub
P
P
IκBα
NF-κB
Degradation
Il6 mRNA
Degradation
Nucleus